

USSN - 09/743,414

REMARKS

This amendment is made to correct the dependency of claim 90 (which was from cancelled claim 1) and to correct typographical errors in claims 82 and 83.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant

By: 

Iver P. Cooper
Reg. No. 28,005

624 Ninth Street, N.W.
Washington, D.C. 20001
Telephone: (202) 628-5197
Facsimile: (202) 737-3528
IPC:lms
G:\ipc\u-z\WHBE\Nielsen4\pto preamend3.wpd

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-72 (cancelled).

73 (previously presented). Recombinant microbial cell comprising

- i) a first enzyme activity controlling assimilation of a nitrogen nutrient source,
wherein the first enzyme activity is encoded by a first nucleic acid operable linked to an expression signal not natively associated with the first nucleic acid, and
wherein the expression of the first enzyme activity is increased as compared to the expression of the first enzyme activity when the first nucleic acid is associated with its native expression signal,

and/or

- ii) a second enzyme activity controlling assimilation of a nitrogen nutrient source,
wherein the second enzyme activity is encoded by a second nucleic acid operable linked to an expression signal not natively associated with the second nucleic acid, and
wherein the expression of the second enzyme activity is increased as compared to the expression of the second enzyme activity when the second nucleic acid is associated with its native expression signal,

the cell further comprising

- iii) a reduced or eliminated expression of a third enzyme activity encoded by a third nucleic acid and controlling assimilation in the cell of a nitrogen

nutrient source, wherein the expression of the third enzyme activity is reduced or eliminated as compared to the expression of the third enzyme activity when the third nucleic acid is associated with its native expression signal.

74 (previously presented). Microbial cell according to claim 73, the cell comprising

- i) a further enzyme activity, the further enzyme activity mediates an energy yielding first reaction resulting in a production of a first metabolite, wherein
- ii) the first reaction being operably linked to an energy requiring second reaction resulting in assimilation of a nutrient source.

75 (previously presented). Microbial cell according to claim 74, wherein the energy requiring second reaction resulting in assimilation of a nutrient source is controlled at least by the first and/or second enzyme activity.

76 (previously presented). Microbial cell according to claim 73, the cell being selected from the group consisting of a fungal cell, a yeast cell, and a bacterial cell.

77 (previously presented). Microbial cell according to claim 76, the cell being a yeast cell.

78 (previously presented). Microbial cell according to claim 73, wherein the nitrogen source is ammonia and/or an ammonium ion.

79 (previously presented). Microbial cell according to claim 73, wherein at least one of the first and second enzyme activities is mediating a biosynthetic reaction.

80 (previously presented). Microbial cell according to claim 73, wherein the first enzyme activity is a glutamate synthase activity.

81 (previously presented). Microbial cell according to claim 80 wherein the activity is a *Saccharomyces cerevisiae*

glutamate synthase, or a functionally equivalent activity capable of catalysing a glutamate synthase reaction.

82 (currently amended). Microbial cell according to claim 73 wherein the second enzyme activity is a glutamine ~~glu-tamine~~ synthetase activity.

83 (currently amended). Microbial cell according to claim 82 wherein the activity is a *Saccharomyces cerevisiae* glutamine synthetase activity, or a functionally equivalent activity capable ~~ca-pable~~ of catalysing a glutamine synthetase reaction.

84 (currently amended). Microbial cell according to claim 73 wherein the third enzyme activity is a glutamate ~~gluta-mate~~ dehydrogenase activity.

85 (previously presented). Microbial cell according to claim 84 wherein the activity is a *Saccharomyces cerevisiae* glutamate dehydrogenase activity, or a functionally equivalent activity capable of catalysing a glutamate dehydrogenase reaction.

86 (previously presented). Microbial cell according to claim 73, wherein the increased expression of the first and/or second enzyme activity encoded by the first and/or second nucleic acid, respectively, results in an increased production of a first metabolite, the production being increased as compared to the production of the metabolite in a cell wherein the first and/or second nucleic acid is associated with a native expression signal.

87 (previously presented). Microbial cell according to claim 86 wherein the first metabolite is ethanol.

88 (previously presented). Microbial cell according to claim 86, wherein the yeast cell further produces a second metabolite, the production of the second metabolite being decreased as compared to the production of the metabolite in a cell wherein the first and/or second nucleic acid is associated with a native expression signal.

89 (previously presented). Microbial cell according to claim 88, wherein the second metabolite is glycerol.

90 (withdrawn; currently amended). Method of producing a first metabolite, the method comprising the steps of

- i) cultivating the microbial cell according to claim ~~±~~ 73 in a suitable growth medium and under such conditions that the microbial cell is producing a first metabolite, and optionally
- ii) isolating the first metabolite in a suitable form, and further optionally
- iii) purifying the isolated first metabolite.

91 (withdrawn). Method of claim 90, wherein the production of the first metabolite is increased in a cell wherein the expression of the first and/or second enzyme activity encoded by the first and/or second nucleic acid, respectively, is increased, as compared to the production of the first metabolite in a cell wherein the first and/or second nucleic acid is associated with a native expression signal.

92 (withdrawn). Method of claim 90, wherein the yeast cell further produces a second metabolite, the production of the second metabolite is decreased as compared to the production of the second metabolite in a cell wherein the first and/or second nucleic acid is associated with a native expression signal.